Cortisone-induced small RNP tightly bound to chromatin

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The small nuclear RNP (α-RNP) tightly bound to chromatin has been isolated. α-RNP can be removed from chromatin together with the acid-soluble proteins. The RNA from this RNP has been isolated; its electrophoretic mobility is equal to that of 4 S RNA. The study of the resistance of α-RNA to RNases (A, T_1 and S_1) in salt solutions of various ionic strengths allows us to conclude that the α-RNA has a well-developed secondary structure. The α-RNA is tightly associated with the protein moiety of α-RNP and has a high metabolic activity.

Small nuclear RNP *Chromatin* *Cortisone induction* *Small RNA* *Hormone acceptor*

1. INTRODUCTION

It is widely known that glucocorticoids induce specific changes in the transcription pattern in rat liver cells: the population of the RNA is changed under the action of cortisone [1,2], the transcription of repetitive sequences being preferentially activated [3,4], and the template activity of chromatin increased [3,5].

Despite much effort to elucidate the precise mechanism by which glucocorticoid hormones induce specific activation of transcription, knowledge of these events remains quite limited. Information about the nature of hormone-binding receptors and acceptors in nuclei is contradictory [6–10]. Thus, the hormone–acceptor interaction has been reported to occur with the chromatin proteins [6], nuclear matrix [7] and DNA [9,10].

It was shown [11] that cortisone induces an increase in the content of the specific non-histone protein (α-protein) in the chromatin of rat liver nuclei. The ^{3}H-labelled hydrocortisone, after in vivo injection, is bound to this protein [12], so the latter can be regarded as the nuclear acceptor of the hormone.

Here, it is shown that the α-protein is the protein moiety of a small RNP, tightly bound to chromatin. The RNA has been isolated from this RNP; its resistance to RNases has been studied. Some data on the properties and the amino acid composition of the α-protein are presented elsewhere [12].

2. MATERIALS AND METHODS

Male Wistar rats (100–120 g) were used. Cortisone-acetate in 1 ml of 0.14 M NaCl (5 mg/100 g body wt) was injected intraperitoneally 3 h before the animals were killed. Control rats received 1 ml of 0.14 M NaCl. [^{14}C]Orotic acid (100 μCi/100 g body wt) was administered 40 min before killing. All animals had been fasting for 24 h. Nuclei, chromatin and acid-soluble chromatin proteins were isolated and purified as in [11]. The RNA was isolated from α-RNP as follows. The acid-soluble chromatin proteins were separated by acetic acid–urea electrophoresis in polyacrylamide gel [13]. The α-RNP was extracted from the band of the gel and treated with pronase (300 μg/ml) in 0.14 M NaCl or proteinase K (250 μg/ml) in 0.2% SDS–0.01 M Tris–HCl (pH 7.0) at 37°C for 3 h. Afterwards it was treated with phenol-chloroform in the presence of 0.5% SDS and RNA was precipitated with ethanol and cetyltrimethylammonium bromide [14]. The resulting RNA was subjected to electrophoresis in

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10% polyacrylamide gel containing 0.2% SDS at pH 7.7 [14]. Pyronin G was used as a marker. The gels were fixed in 50% ethanol and stained with ethidium bromide (2 μg/ml, 30 min).

To study the sensitivity of the α-RNA to RNases, the RNase A was used at a concentration of 50 μg/ml; RNase T_1, 50 units/ml; S_1 nuclease, 3 units/ml. The treatment by S_1 nuclease was performed in 0.01 mM $ZnSO_4$–0.02 M Na acetate (pH 4.6)–0.3 M NaCl at 37°C for 45 min. The reaction was stopped by 5 mM EDTA.

Reagents: [^{3}H]orotic acid, 1 Ci/mmol (Amersham, England); [^{14}C]orotic acid, 63 mCi/mmol ('Isotop', USSR); RNase A, T_1, S_1 and pronase (Calbiochem, USA); pyronin G (Merck, FRG); proteinase K (Boehringer, FRG).

3. RESULTS AND DISCUSSION

As shown previously [11], the α-protein, the content of which is increased under the action of cortisone, moves slower than H1 histone on electrophoresis in the acetic-urea system, and in the SDS system it migrates between H1 and core histones (fig.1).

After in vivo injection, the [^{14}C]orotic acid incorporates predominantly into the electrophoretic fraction corresponding to the α-protein (fig.2). On the basis of this observation, it was assumed that the α-protein is associated with RNA. But it is also possible that RNA can nonspecifically bind to the chromatin proteins during the chromatin isolation procedure.

To test this possibility, the ^{3}H-labelled heterogeneous nuclear RNA (specific radioactivity 5000 cpm/μg) was added to nuclear suspension before chromatin isolation. Afterwards, the chromatin was isolated as usual and acid-soluble proteins were extracted and subjected to electrophoresis in polyacrylamide gel. No radioactivity was observed in these gels. Thus, the incorporation of the [^{14}C]orotic acid into α-band is not due to unspecific binding of RNA to proteins during chromatin isolation. Similarly, no co-migration of RNA was observed after the addition of [^{14}C]RNA directly to the samples before the electrophoresis.

This implies that the α-protein is associated with RNA. The α-RNP is extracted together with acid-soluble proteins, and we suggest that it is acid-soluble because the RNA component is packed in-

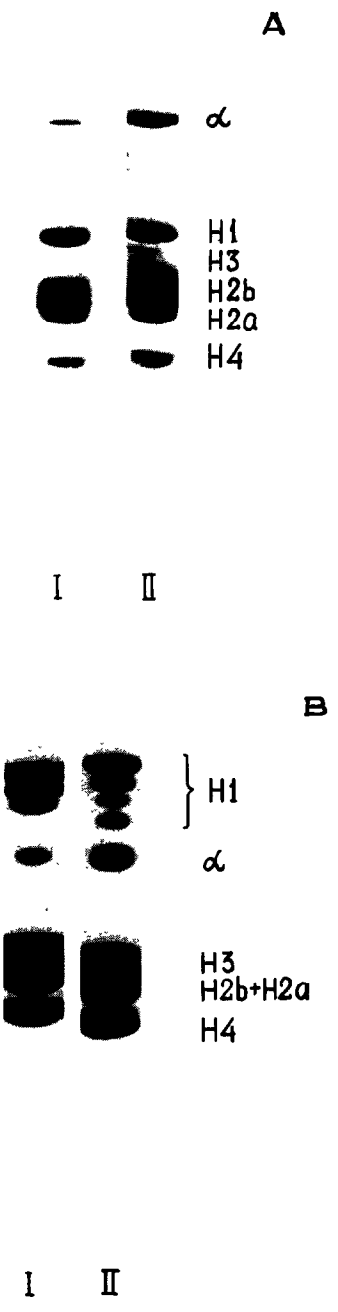

Fig.1. Electrophoresis of the acid-soluble proteins of the chromatin of rat liver nuclei in 12% polyacrylamide gel. (A) Electrophoresis was done in 0.9 N acetic acid with 6 M urea for 3.5 h at 150 V. (B) Electrophoresis was carried out in the presence of 1% SDS with Tris–glycine buffer, pH 8.3. (I) Proteins from the livers of control rats; (II) proteins isolated from the livers of cortisone-treated rats.

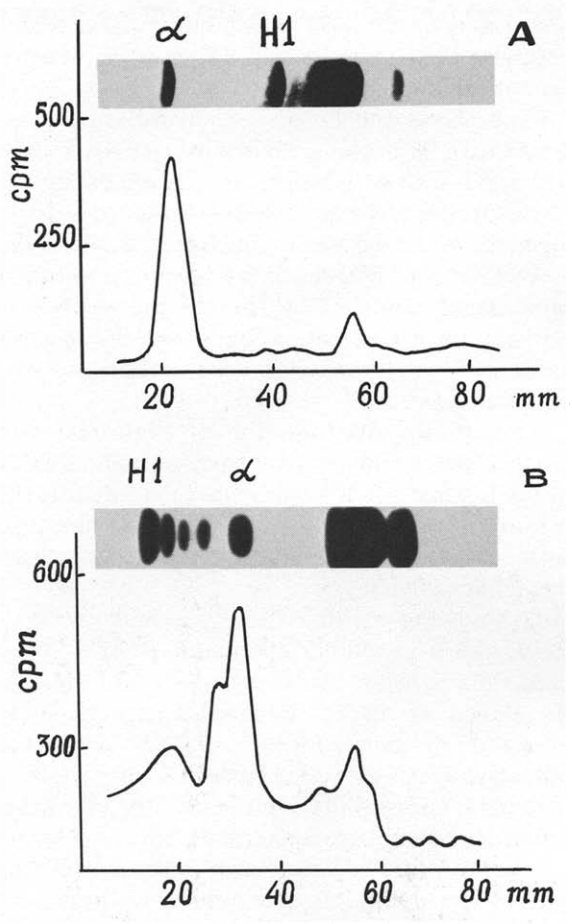

Fig.2. Distribution of the [^{14}C]orotic acid in the fractions, corresponding to acid-soluble chromatin proteins. Conditions of the electrophoresis are as in fig.1 (A,B). Abscissa, distance from the starting point. Ordinate, radioactivity (cpm).

side the RNP particle, and the positively charged proteins are exposed outside. This suggestion is supported by the results of the RNase digestion: the α-RNA within the α-RNP is protected by protein from the nuclease attack: it is not sensitive to the action of RNases, and becomes RNase-sensitive only after deproteinization.

The α-RNP can move in the acetic acid gel electrophoresis (fig.1A), which suggests that the net charge of the α-RNP particle is positive. We propose that the positive groups of the protein component can neutralize the negative charges of the α-RNA. Besides, at pH 2.5 nitrogen bases of the RNA are protonated.

The α-RNA was isolated from the electrophoretic band, as described in section 2. The α-RNA preparations, purified without extensive pronase or proteinase treatment, contained high amounts of protein contaminants ($E_{260}/E_{230} < 1$). To isolate pure RNA, an extensive proteinase treatment was needed.

Electrophoresis of the isolated native α-RNA was performed in 10% polyacrylamide gel. As can be seen from fig.3, α-RNA is a low-M_r RNA, its electrophoretic mobility being close to that of 4 S RNA. Minor components can also be seen (fig.3). Therefore, the M_r of the α-RNA is about 20000. However, the apparent M_r of the total α-RNP particle in SDS–gel electrophoresis is lower than that of H1 histone (<23000). It seems contradictory, but the α-RNP does not move according to its molecular mass in SDS–gel electrophoresis, because SDS neutralizes the positive charges of the proteins, and the RNA remains negatively charged. Thus, no conclusions concerning the molecular

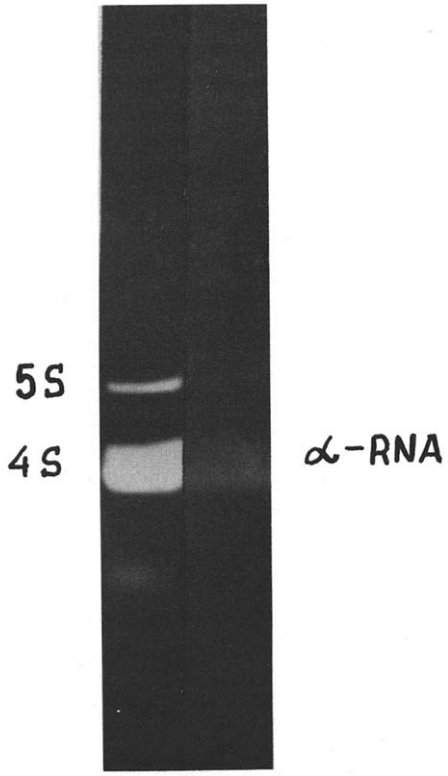

Fig.3. Electrophoresis of the native α-RNA in 10% polyacrylamide in the presence of 0.2% SDS at pH 7.7.

mass of the α-RNP can be drawn on the basis of its electrophoretic mobility in the SDS gel.

The sensitivity of the α-RNA to RNases is illustrated in table 1. After denaturation (100°C, 10 min), the sensitivity of α-RNA to RNases A and T_1 in 0.35 M NaCl and to nuclease S_1 is increased. Both treatments are known to destroy the single-stranded RNA regions. Thus, it can be suggested that a part of the α-RNA is double-stranded and becomes single-stranded and RNase-sensitive after denaturation.

Table 1

Resistance of α-RNA to ribonucleases

State of [^{3}H]-α-RNA	RNases	Resistance to RNases (%)
Native	A + T_1 (in 0.35 M NaCl)	75
Native	S_1	70
Denatured	A + T_1 (in 0.35 M NaCl)	30
Denatured	S_1	28

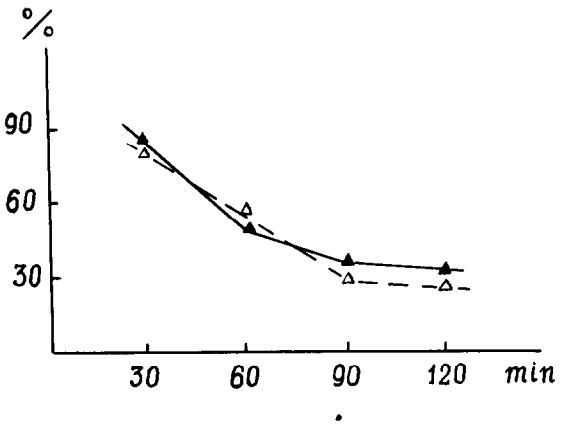

Fig.4. Digestion by (A + T_1) RNases of denatured α-RNA molecules isolated from chromatin of normal and cortisone-stimulated rat livers. Orotic acid-labelled α-RNA was prepared as described in section 2 and samples containing 5000 cpm were dissolved in 0.14 M NaCl and treated at 37°C with (A + T_1) RNases for the indicated times. Acid-precipitable radioactivity is plotted. (▲—▲) Control RNA, (△---△) RNA isolated after cortisone treatment.

Fig.4 shows the kinetics of hydrolysis of the denatured [^{3}H]orotic acid-labelled α-RNA by the RNases in 0.14 M NaCl. Under these conditions, single-stranded as well as double-stranded RNA is digested. As can be seen in this figure, a part of the α-RNA (about 25%) remains RNase-resistant even after denaturation. This part of the α-RNA is possibly protected by peptide which remains associated with it even after denaturation and proteinase treatment.

After the in vivo injection of [^{3}H]orotic acid (50 μCi/100 g body wt) the specific radioactivity of the isolated α-RNA was higher than that of the heterogeneous nuclear RNA (3000 *vs* 500 cpm/μg). This implies that the α-RNA has a very high metabolic activity.

To study the possible tissue specificity of the α-RNP, the acid-soluble chromatin proteins have been isolated from two tissues: liver and thymus. As shown in fig.2, ^{14}C-labelled material was observed in the zone of α-RNP after the polyacrylamide gel electrophoresis of the liver chromatin acid-soluble proteins. In the acid-soluble chromatin proteins from thymus, no incorporation of the [^{14}C]orotic acid in the α-RNP has been observed, though some protein was present in this electrophoretic zone. Thus, the presence of the chromatin is tissue specific (for these two tissues). As the action of the hormones is tissue specific, it may be assumed that the α-RNP may be involved in the specific cellular responses to hormonal stimuli.

The α-RNP is tightly bound to chromatin, it cannot be extracted from chromatin with NaCl solutions of ionic strength up to 1.0 M. Among various data regarding steroid acceptors [6–10], there have been reports that nuclear and cytoplasmic 50 S and 30 S RNP particles bind steroid–receptor complexes [8]. Unlike α-RNP, these particles are not bound to chromatin and contain heterogeneous RNA [8]. The tight binding of α-RNP to chromatin, the increase of its content in chromatin after hormone treatment, and its tissue specificity allow us to suggest that this RNP has a regulatory function, changing specificity of transcription. Further studies are in progress to elucidate its role.

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